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Differential gene expression in soybean infected by *Phakopsora pachyrhizi* in response to acibenzolar-S-methyl, jasmonic acid and silicon

Maria Fernanda Antunes Cruz[1] | Marcos Oliveira Pinto[2] | Everaldo Gonçalves Barros[3] | Fabrício Ávila Rodrigues[4]

[1]Universidade Federal do Pampa, Itaqui, RS, Brazil

[2]Embrapa Milho e Sorgo, Sete Lagoas, MG, Brazil

[3]Universidade Federal de Viçosa, Departamento de Biologia Geral, Viçosa, MG, Brazil

[4]Laboratório da Interação Planta-Patógeno, Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, MG, Brazil

Correspondence
Fabrício A. Rodrigues, Laboratório da Interação Planta-Patógeno, Departamento de Fitopatologia, Universidade Federal de Viçosa, Viçosa, MG, Zip Code 36570-900, Brazil.
Email: fabricio@ufv.br

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Abstract

Asian soybean rust (ASR), caused by *Phakopsora pachyrhizi*, has negatively impacted soybean production worldwide. This study evaluated the effect of foliar spray of acibenzolar-S-methyl (ASM) and jasmonic acid (JA) as well as silicon (Si) supplied, either by soil amendment using calcium silicate or through a nutrient solution, on ASR control as well as on the expression of some defence-related genes (phenylalanine ammonia-lyase (*PAL*), chitinase (*CHI*), chalcone isomerase (*CHAL*), lipoxygenase (*LOX*), pathogenesis-related protein 1 (*PR-1*) and metalloproteinase (*MET*)) in soybean plants infected or not by *P. pachyrhizi*. Foliar Si concentration ranged from 2.6 to 8.7 dag/kg for Si supply by soil application or through a nutrient solution in comparison with the control treatment (no Si supply). Higher foliar Si concentration contributed to reducing ASR severity greater than 30%, mainly if supplied through nutrient solution. The ASR severity was significantly reduced by 36% with the ASM spray in comparison with the control treatment without any expressive effect on the expression of the analysed genes. The JA spray did not result in reduction on ASR severity in comparison with the control treatment even though with higher transcript levels of *PAL* at 12 hai. The transcripts levels of *MET* and *PAL* were significantly higher for inoculated plants taking up Si from the soil in comparison to non-inoculated plants of this treatment at 72 hai. For Si supply through the nutrient solution (+Si plants), the transcripts levels of *LOX* and *CHAL* were significantly higher for inoculated plants in comparison to inoculated -Si ones at 12 hai. At 141 hai, the transcript level of *PAL* was significantly higher for inoculated + Si plants in comparison to inoculated -Si ones. In conclusion, Si supply contributed decisively to reduce ASR severity through the potentiation of some defence-related genes mainly *PAL* and *CHAL* involved, respectively, in the biosynthesis of phenolics and flavonoids.

KEYWORDS

Asian soybean rust, expression of defence genes, induced resistance, plant hormones, plant nutrition

1 | INTRODUCTION

Soybean production has been greatly impacted by worldwide epidemics of Asian soybean rust (ASR), caused by *Phakopsora pachyrhizi* Syd. & P. Syd (Reis et al., 2006). The fungal infection process starts with urediniospores deposition on the leaf surface where they germinate and form germ tubes and appressoria that directly penetrate the host cuticle (Bonde et al., 1976). Fungal penetration takes place around 22 hr after inoculation and fungal hyphae colonize the intercellular space of epidermal and mesophyll cells forming haustoria inside them (Bonde et al., 1976; McLean, 1979). Eight days after inoculation, fungal mycelia give rise to uredinia (Marchetti et al., 1975). Urediniospores are differentiated within the uredinia between 8 and 10 days after fungal penetration and are released to the environment after their maturation to start autoinfections or alloinfections (Marchetti et al., 1975, Reis et al., 2006).

The ASR dramatically reduces the photosynthetically active leaf area and causes severe plant defoliation and, consequently, contributes to reducing the number of pods per plant as well as the number and quality of the seeds (Reis et al., 2006). The use of resistant cultivars, crop rotation and fungicide sprays are the control strategies currently used for ASR management (Reis et al., 2006). However, the lower sensitivity of *P. pachyrhizi* to fungicides has led to a greater number of applications and yield reduction with increasing the selection pressure of fungicides on pathogen populations (Godoy et al., 2016, Langenbach et al., 2016). The use of plant activators that induce resistance either through systemic acquired resistance (SAR) or induced systemic resistance (ISR) pathways arises as an alternative to reduce the number of fungicides applications (Durrant & Dong, 2004). The SAR is characterized by activation of the salicylic acid-dependent pathway while the ISR is activated through the route mediated by jasmonic acid (JA) and ethylene (Dong, 1998; Reymond & Farmer, 1998; Durrant & Dong, 2004). In addition to the use of plant activators that induce resistance aiming to control ASR (Cruz et al., 2014; Twizeyimana & Hartman, 2019), silicon (Si) also shows great potential. The intensity of several foliar and root diseases occurring in both dicots and monocots has been reduced by Si supply to the plants (Debona et al., 2017; Rodrigues et al., 2015). Reduction on disease symptoms is attributed to the formation of a physical barrier provided by Si deposition beneath the cuticle that avoids or delays fungal penetration (Kim et al., 2002) or through the potentiation of host defence responses such as an increase in the concentrations of phenolics, lignin and phytoalexins as well as greater activation of defence genes such as glucanase, peroxidase and PR-1 (Debona et al., 2017; Rodrigues et al., 2015). According to Arsenault-Labrecque et al. (2012), plants from soybean cultivar Hikmok sorip accumulated nearly four times more foliar Si concentration than plants from cultivar Williams 82 and showed lower ASR severity. The area under ASR progress curve was significantly reduced by 43% and 36%, respectively, for plants grown in soil amended or sprayed with Si (Lemes et al., 2011).

Considering the need to find new strategies for ASR control, the present study investigated the potential of using acibenzolar-S-methyl, JA, and Si (supplied either by soil amendment with calcium silicate or through nutrient solution) on the potentiation of soybean resistance against *P. pachyrhizi* infection by examining the differential expression of genes commonly related to host defence responses.

2 | MATERIAL AND METHODS

2.1 | Experiment 1: Soil amendment with calcium silicate, plant growth and treatments

Plastic pots were filled with air-dried and sieved (5 mm) soil (2 kg per pot) belonging to a typical Acrustox red–yellow latosol. The concentration of available Si (extraction in $CaCl_2$) was of 12 mg/dm^3 indicating its deficiency in Si. The physical and chemical characteristics of this soil were reported by Rezende et al. (2009). The Si was obtained from calcium silicate (AgroSilício®, 10.5% Si, 25% Ca, and 6% Mg; Harsco Minerais Ltda, Timóteo, Minas Gerais, Brazil). Each pot received 0 and 1.75 g/kg (0 and 0.39 g of Si, respectively) of calcium silicate. The amounts of Ca and Mg in calcium silicate were equilibrated among treatments using calcium carbonate and magnesium carbonate according to Rezende et al. (2009).

After 60 days of incubation, soybean seeds from cultivar MG/BR 46 (Conquista), susceptible to ASR, were sowed at each pot and thinned to two seedlings after emergence. Plants in each pot were fertilized weekly using a nutrient solution described by Cruz et al. (2014). The treatments used in the experiment were as follows: (a) deionized water spray (control), (b) acibenzolar-S-methyl (ASM) spray (0.4 g/L) (Bion® 500 WG Syngenta Crop Protection Inc.), (c) JA spray (2 mM) (Sigma-Aldrich, São Paulo, Brazil) at 24 hr before inoculation with *P. pachyrhizi* and (d) soil amended with calcium silicate. A VL Airbrush atomizer (Paasche Air-brush Co.) was used to spray deionized water as well as the solutions of ASM and JA (25 ml per plant) until runoff.

2.2 | Experiment 2: Nutrient solution preparation, plant growth and treatments

The monosilicic acid (2 mM) was obtained following the procedures of Domiciano et al. (2015). Plants were grown in a nutrient solution as described by Domiciano et al. (2015). In the beginning, plants remained in plastic pots with one-half strength nutrient solution without Si for two days and transferred to new plastic pots containing 5 litres of nutrient solution, without or with Si, thereafter.

2.3 | Plant inoculation with *P. pachyrhizi*

Plants (V6 growth stage) (Fehr & Caviness, 1977) were inoculated with a suspension of urediniospores of a monopustular isolate of *P. pachyrhizi* (UFV-DFP *Pp*-16) at a concentration of 1×10^5

TABLE 1 Primer sequences for the genes phenylalanine ammonia-lyase (*PAL*), chitinase (*CHI*), chalcone isomerase (*CHAL*), lipoxygenase (*LOX*), pathogenesis-related protein 1 (*PR-1*), metalloproteinase (*MET*), portion of the 18S ribosomal RNA of *Phakopsora pachyrhizi* (*RUST*), *Cons7* and *Ubiquitin-3* analysed by quantitative reverse transcription PCR on the leaves of soybean plants inoculated with *P. pachyrhizi* and submitted to different treatments

Genes	Phytozome or Genbank	Primer forward 5'-3'	Primer reverse 5'-3'
PAL	Glyma03g338901	ACGGTTCATTTTGCTTGTCC	ACCTGAGCGATGGTGAGAGT
QUI	Glyma02g048201	TTCTTGGCTCAAACTTCTCATA	CCCACGCATATGGACCATCT
CHAL	Glyma20g385701	GTTTCCCCTGCTTTGAAAGAGA	GGATTGGCCTCTAACTCTTTGAAG
LOX	Glyma13g423401	ACAAGCTAGGCACAACAAAAA	TTGTTCCTCCGATGATTCCAA
PR-1	AF136636.1	GCACTACACACAGGTCGTTTGG	CCTCCGTTATCACATGTCACTTTG
MET	Glyma01g04350	TGGGCTCTTCCCAGTGAAA	TTGCCGCACTCTCCAAGTC
RUST	EF560586.1	ATTCGAAGCCGGTATTTCTAAG	CCACTTGGTTGTGTCCATCTTAT
Cons 7	IDAW310136	ATGAATGACGGTTCCCATGTA	GCATTAAGGCAGCTCACTCT
Ubiquitin 3	Glyma20g27950.1	GTGTAATGTTGGATGTGTTCCC	ACACAATTGAGTTCAACACAAACCG

urediniospores/ml following the procedures described by Cruz et al. (2014). After inoculation, plants remained in a mist chamber (temperature of 24 ± 2°C and relative humidity of 90 ± 5%) for 24 hr in the dark. After this period, plants were transferred to a plastic mist growth chamber (temperature of 28 ± 4°C (day) to 22 ± 2°C (night) and relative humidity of 92 ± 3%) with natural photon flux density.

2.4 | Assessment of ASR severity

A diagrammatic scale (Godoy et al. 2006) was used to assess ASR severity in the fourth, fifth and sixth leaves, from base to top, of each plant per replication (four replications and 20 plants total) of each treatment at 18 days after inoculation (dai).

2.5 | Determination of foliar calcium (Ca) and Si concentrations

The Si concentration was determined on dried and ground leaf tissues collected at 19 dai (20 plants and 60 leaves total per treatment) according to the methodology proposed by Korndörfer et al. (2004). The Ca concentration was determined by atomic absorption spectrophotometry in solution of leaf tissues digested with a nitric-perchloric solution (3:1, v/v) at 19 dai.

2.6 | RNA isolation and quantitative RT-PCR analysis

The fourth, fifth and sixth leaves, from base to top, of each plant per replication of each treatment (four replications and 20 plants per each sampling time) were collected at 12, 72 and 141 hr after inoculation (hai). Leaves were stored in aluminium foil, rapidly frozen in liquid nitrogen and stored in an ultra freezer at −80°C until further analysis. Leaf samples were ground to a fine powder for total RNA extraction using liquid nitrogen and Brazol reagent (LGC Biotechnology Ltd.) according to the manufacturer's instructions. Total RNA was extracted from a total of 100 mg of leaves per replication of each treatment at each evaluation time from two different experiments. Three replicates were used per each sample. After extraction, total RNA concentration was quantified in a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Rockland, USA) and the RNA was assessed for its quality based on the integrity of the ribosomal RNA bands on 1.5% agarose gel. The ratio of absorbance at 260 and 280 nm was used for RNA purity determination. Total RNA was treated with RNase Free DNase (Promega, São Paulo, Brazil) following quantification and evaluation of integrity. Synthesis of the first cDNA strand used 1 μg of total RNA, M-MLV reverse transcriptase (Invitrogen, São Paulo, Brazil) and the primer oligo (dT) 12-18 (Sigma-Aldrich) according to the manufacturer's instructions. The genes encoding for phenylalanine ammonia-lyase (*PAL*), chitinase (*CHI*) (Nogueira, 2007), chalcone isomerase (*CHAL*) (Panthe et al., 2007), lipoxygenase (*LOX*) (Brito Júnior, 2007), pathogenesis-related protein 1 (*PR-1*) (Nogueira, 2007) and metalloproteinase (*MET*) (Nogueira, 2007) were chosen for this study because of their importance for host resistance against pathogens attack. The primer sequences for amplification of the *CHI*, *CHA*, *LOX*, *PR-1* and *MET* genes were obtained from the literature. In the case of *PAL* and the portion of the 18S ribosomal RNA of *P. pachyrhizi* (named *RUST*), primers were designed using the Primer Express software (Applied Biosystems, Foster City, USA) and confirmed following the sequences deposited in the data bank Phytozome (http://www.phytozome.net/soybean) for *Glycine max* or NCBI (http://www.ncbi.nlm.nih.gov/) for *P. pachyrhizi*. The qPCR primer sequences for the genes investigated are listed in Table 1. The $2^{-\Delta Ct}$ method was used for the relative quantification of transcripts (Livak, 2001). The expressions of the constitutive genes *Cons7* (Libault et al., 2008) and *Ubiquitin-3* (Mortel et al., 2007) were used to normalize the accumulation of their transcripts. qPCR reactions contained 90 ng cDNA template, 10 μl 2 × SYBR-green master PCR mix (Applied Biosystems) and primers at a concentration of 200 nM for each forward and reversed primers. Amplification conditions were as follows: one step of 50°C for 2 min, one step at 95°C for 10 min, 40 cycles at 95°C for 30 s and

60°C for 30 s. Amplification of specific regions of targeted genes and real-time detection of amplicon production was performed in an ABI model 7000 sequence detection system (Applied Biosystems).

2.7 | Experimental design and data analysis

A 4 × 2 factorial experiment (Experiment 1) was arranged in a completely randomized design with sixteen replications. The factors studied were four treatments (control, ASM, JA and calcium silicate) and non-inoculated (NI) and inoculated (I) plants. A 2 × 2 factorial experiment (Experiment 2), consisting of two Si concentrations (0 and 2 mM, hereafter referred to -Si and + Si plants, respectively) and NI and I plants, was arranged in a completely randomized design with sixteen replications. A plastic pot with five plants corresponded to one experimental unit. Experiments 1 and 2 were repeated. A difference of 25 and 28 days occurred between the repetitions of experiments 1 and 2, respectively. Data from experiments 1 and 2, and their respective repetitions, were analysed using the MIXED procedure of the SAS software (version 8.02 for Windows; SAS Institute, Inc.) to determine whether data from the experiments could be combined (Moore & Dixon, 2015). Data were checked for normality and homogeneity of variance and subjected to analysis of variance (ANOVA). Based on the ANOVA of combined data from Experiment 1 and its repetition, the factors treatments (T) ($p = .008$), NI and I plants (P) ($p = .007$), and the interaction T × P ($p = .005$) were significant. The factor experiment (E) and the interactions T × E ($P = .824$), P × E ($p = .568$) and T × P × E ($p = .636$) were not significant. The ANOVA of combined data from Experiment 2 and its repetition showed that the factors Si concentrations (Si) ($p = .012$), P ($p = .018$) and the interaction Si × P ($p = .042$) were significant. The factor experiment (E) and the interactions Si × E ($p = .785$), P × E ($p = .637$) and Si × P × E ($p = .952$) were not significant ($p = .952$). Data were submitted to ANOVA, and treatments means were compared by *F* and Tukey tests ($p \leq .05$) using the SAS software.

3 | RESULTS

3.1 | Effect of Si amended to the soil on ASR control and gene expression

The factor T (control, ASM, JA, and Si) was significant for ASR severity and foliar Si concentration ($p \leq .05$), but not for foliar Ca concentration ($p \geq .05$). The factors T and P as well as the T × P interaction were significant ($p \leq .05$) for *MET*, *LOX*, *PR-1*, *CHAL* and *PAL* expressions.

The ASR severity was significantly reduced by 32 and 36%, respectively, for Si and ASM treatments in comparison to the control treatment (Figure 1). For foliar Ca concentration, there was no significant difference among treatments. The foliar Si concentration was significantly higher (2.6 dag/kg) for Si treatment in comparison to the control treatment (0.6 dag/kg).

The *F* values for the comparisons between NI and I plants for each treatment and gene expression, at each sampling time, are shown in Table S1. At 12 hai, the transcripts levels of *MET*, *PR-1* and *CHAL* were significantly higher for non-inoculated plants from the control, JA and Si treatments, respectively, in comparison to their inoculated counterparts (Figure 2a-b, e-f, and i-j). For non-inoculated plants from JA and ASM treatments, the transcript level of *LOX* was significantly higher in contrast to their inoculated counterparts at 12 hai (Figure 2c-d). There were significant increases for the transcript levels of *CHAL* for the control treatment and of *PAL* for control and JA treatments on inoculated plants in comparison to non-inoculated plants of these same treatments at 12 hai (Figure 2i-j and k-l). The transcripts levels of *MET* and *PAL* were significantly higher for inoculated plants from Si treatment in comparison to non-inoculated plants of this treatment at 72 hai (Figure 2a-b and k-l). At 141 hai, the transcripts levels of *MET* and *PAL* were significantly higher for non-inoculated plants from Si treatment in comparison to their inoculated counterparts (Figure 2 a-b and k-l).

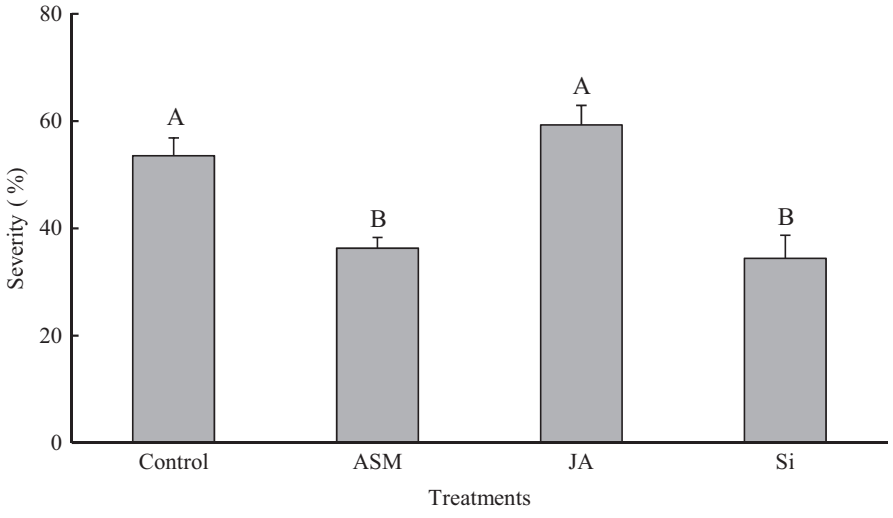


FIGURE 1 Severity of Asian soybean rust on the leaves of soybean plants sprayed with water (control), Acibenzolar-S-Methyl (ASM) and jasmonic acid (JA) as well as grown in soil containing silicon (Si). Means followed by the same letter are not significantly different according to Tukey´s test ($p \leq .05$)

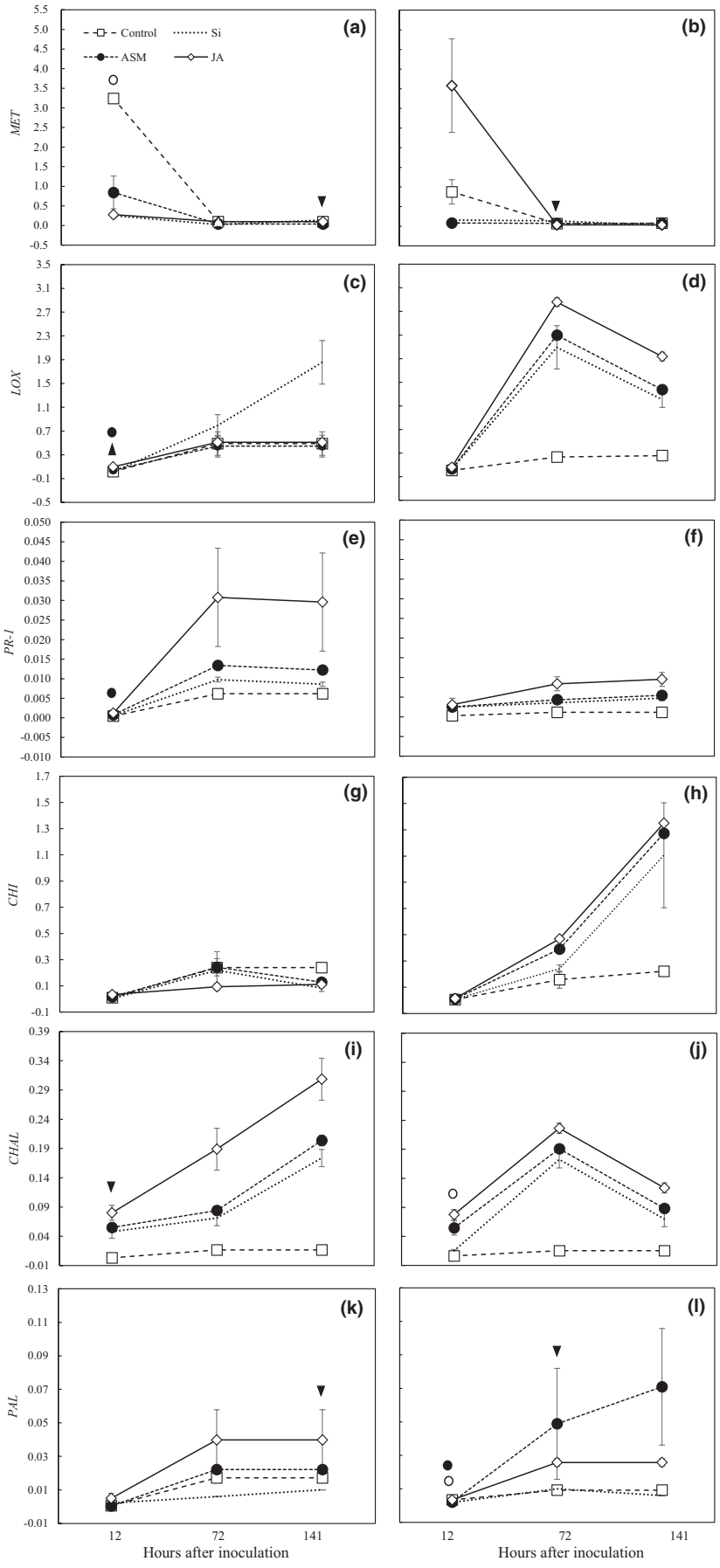


FIGURE 2 Expression levels of the genes coding for metalloproteinase (*MET*) (a and b), lipoxygenase (*LOX*) (c and d), pathogenesis-related protein 1 (*PR-1*) (e and f), chitinase (*CHI*) (g and h), chalcone isomerase (*CHAL*) (i and j) and phenylalanine ammonia-lyase (*PAL*) (k and l) determined in the leaves of soybean plants sprayed with water (control), Acibenzolar-S-Methyl (ASM), and jasmonic acid (JA) or supplied with silicon (Si) that were non-inoculated (NI) (a, c, e, g, i and k) or inoculated (I) (b, d, f, h, j and l) with *Phakopsora pachyrhizi*. Means between NI and I plants, at each sampling time, followed by hollow circle (○), filled circle (●), filled triangle (▲) and inverted filled triangle (▼), respectively, for the control, ASM, JA and Si treatments, are significantly different ($p \leq .05$) based on *F* test. The bars represent the standard errors of the means

3.2 | Effect of Si supplied from nutrient solution on ASR control and gene expression

The factor Si (-Si and + Si plants) was significant for ASR severity, foliar Si concentration, and *LOX*, *CHAL* and *PAL* expressions ($p \leq .05$). The factor P was significant for *MET*, *CHI*, *CHAL* and *PAL* expressions ($p \leq .05$). The Si × P interaction was significant ($p \leq .05$) only for *CHAL* and *PAL* expressions.

The ASR severity was significantly reduced by 60% for + Si plants in comparison to -Si ones. The foliar Si concentration was significantly higher (8.7 dag/kg) for + Si plants in comparison to -Si plants (3.6 dag/kg).

The transcripts levels of *LOX* and *CHAL* were significantly higher for inoculated + Si plants in comparison to inoculated -Si ones at 12 hai (Figure 3c-d and i-j). The transcript level of *CHAL* was significantly higher for inoculated -Si plants in comparison to inoculated + Si plants at 141 hai (Figure 3i-j). The transcript level of *PAL* was significantly higher for -Si plants in comparison to + Si ones at 12 hai regardless of plant inoculation (Figure 3k-l). At 141 hai, the transcript level of *PAL* was significantly higher for inoculated + Si plants in comparison to inoculated -Si ones (Figure 3k-l). The transcript level of *MET* was significantly higher for non-inoculated + Si plants in comparison to inoculated + Si ones at 12 hai (Figure 3a-b). At 12 hai, the transcripts levels of *CHAL* and *PAL* were significantly higher for inoculated + Si plants in comparison to non-inoculated + Si plants (Figure 3i-l). The transcripts levels of *CHI*, *CHAL* and *PAL* were significantly higher for inoculated + Si plants in comparison to non-inoculated + Si plants at 141 hai (Figure 3g-l). At 141 hai, the transcript level of *CHAL* was significantly higher for inoculated -Si plants in comparison to non-inoculated -Si ones (Figure 3i-j). The transcript level of *PAL* was significantly higher for inoculated -Si plants in comparison to non-inoculated -Si ones at 12 hai (Figure 3k-l).

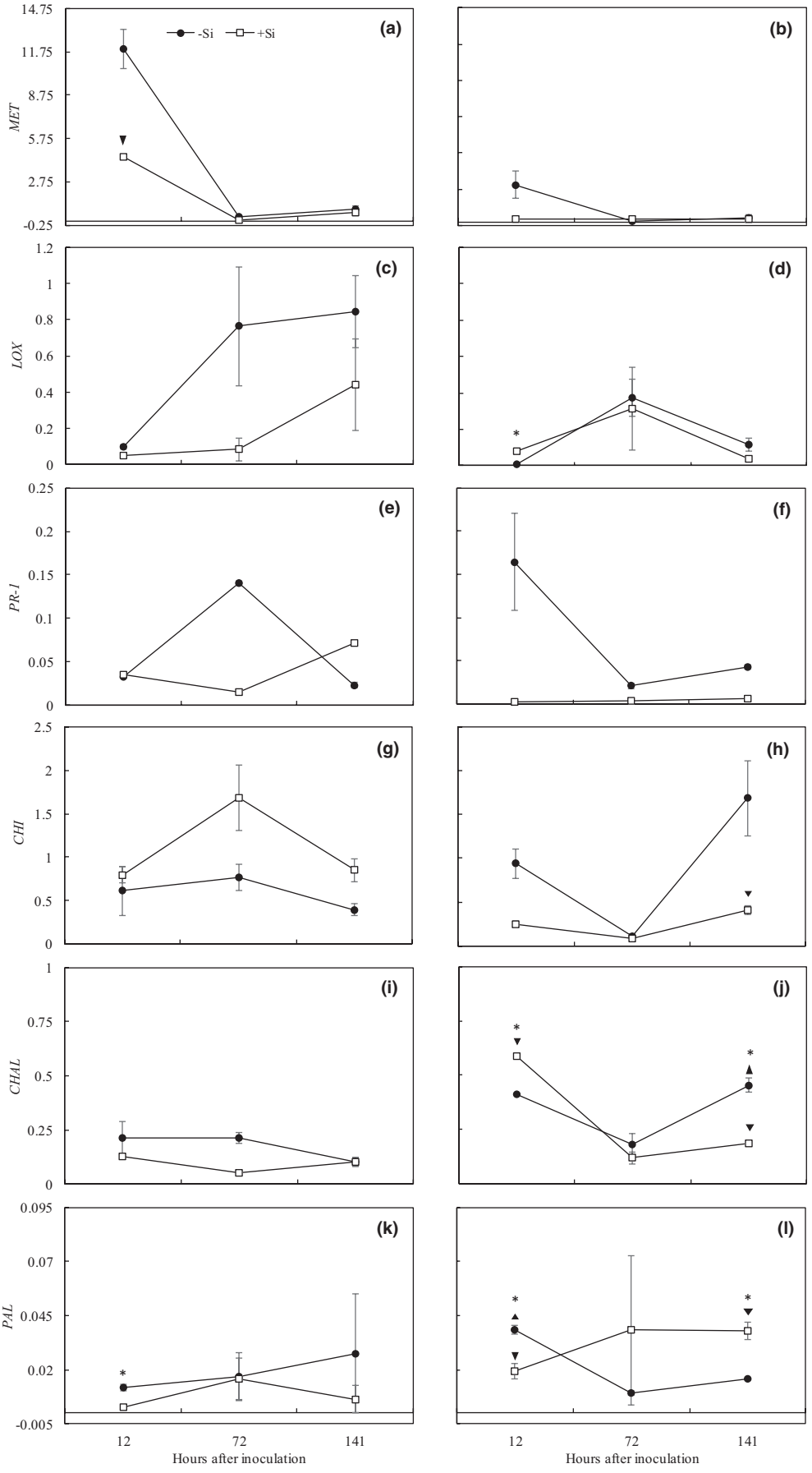
4 | DISCUSSION

Studies of gene expression in soybean against *P. pachyrhizi* infection have been reported in the literature (Mortel et al., 2007; Panthee et al., 2009; Tremblay et al., 2010); however, this study is the first to show the influence of two inducers of resistance and Si on soybean plants on the expression of defence-related genes.

The ASR severity was reduced on the leaves of plants sprayed with ASM and also supplied with Si. Although the use of calcium silicate seemed to be effective in reducing ASR symptoms, Si supply through nutrient solution resulted in a much greater reduction of ASR severity due to high expression of defence-related genes in response to *P. pachyrhizi* infection. The positive effect of Si in reducing ASR symptoms has been reported (Pereira et al., 2009; Rodrigues et al., 2009; Lemes et al., 2011; Arsenault-Labrecque et al., 2011). Reduction of ASR severity due to ASM treatment was linked to a longer incubation period, fewer lesions and fewer uredia produced per lesion (Cruz et al., 2014). Twizeyimana & Hartman (2019) reported that ASM reduced the sporulation of *P. pachyrhizi* on soybean leaves more than treatment with *Trichoderma harzianum*, harpin protein and neem oil. Moreover, ASR severity was lower on the leaves of soybean plants sprayed with potassium silicate or grown in a Si-deficient soil amended with calcium silicate (Cruz et al., 2014). According to Cruz et al. (2013), uredia on the leaves of soybean plants sprayed with potassium silicate or supplied with Si through the roots were smaller and more compact than those observed on the control plants. The polymerization of Si below the cuticle of rice leaves avoids or delays the penetration by *Pyricularia oryzae* and resulted in fewer blast lesions (Kim et al., 2002). Differential regulation of genes occurred in rice plants supplied with Si when infected by *P. oryzae* (Brunings et al., 2009). Moreover, Si had a positive effect on rice metabolism rather than acting only as a physical barrier against fungal infection (Brunings et al., 2009). In comparison to rice, soybean plants are not efficient in Si translocation from the soil solution to shoots and this response is genotype-dependent (Arsenault-Labrecque et al., (2012). In the present study, plants from cultivar MG/BR 46 were very responsive to Si supply either by soil amendment or nutrient solution resulting in foliar Si concentration greater than 2 dag/kg.

The differential *PAL* expression in the infected plants supplied with calcium silicate contributed to reducing ASR severity indicating a participation of the phenylpropanoid pathway. In this pathway, *L*-phenylalanine is converted to trans-cinnamic acid by PAL with the production of phenolics, phytoalexins and lignin (Campbell & Sederoff, 1996). PAL activity in soybean leaves supplied with calcium silicate increased in response to *P. pachyrhizi* infection at 72 hai (Cruz et al., 2013) corroborating with the findings of the present study that showed a peak on *PAL* expression also at 72 hai. In comparison to the JA treatment, there was a delay on *PAL* expression for the infected plants supplied with calcium silicate. Mortel et al. (2007) reported differential changes in gene expression within the first 12 hai for plants from resistant and susceptible soybean genotypes infected with *P. pachyrhizi*, especially those related to phenylpropanoid pathway. For the resistant genotype, gene expression diverged from non-inoculated plants at 72 hai, indicating earlier activation of host defence responses (Mortel et al., 2007).

FIGURE 3 Expression levels of the genes coding for metalloproteinase (*MET*) (a and b), lipoxygenase (*LOX*) (c and d), pathogenesis-related protein 1 (*PR-1*) (e and f), chitinase (*CHI*) (g and h), chalcone isomerase (*CHAL*) (i and j), and phenylalanine ammonia-lyase (*PAL*) (k and l) determined in the leaves of soybean plants grown in hydroponic culture containing 0 (-Si) or 2 mM (+Si) silicon (Si) and non-inoculated (a, c, e, g, i and k) or inoculated (b, d, f, h, j and l) with *Phakopsora pachyrhizi*. The expression levels of *PR-1* and *CHI* were multiplied by 10 for non-inoculated plants. Means for -Si and + Si treatments, within each sampling time, followed by an asterisk (*) are significantly different ($p \leq .05$) based on F test. Means for non-inoculated and inoculated treatments, within each sampling time, followed by filled triangle (▲) and filled inverted triangle (▼) for -Si and + Si, respectively, are significantly different ($p \leq .05$) based on *F* test. The bars represent the standard errors of the means


(a)
-Si
+Si
MET
14.75
11.75
8.75
5.75
2.75
-0.25
(b)
(c)
LOX
1.2
1
0.8
0.6
0.4
0.2
0
(d)
*
(e)
PR-1
0.25
0.2
0.15
0.1
0.05
0
(f)
(g)
CHI
2.5
2
1.5
1
0.5
0
(h)
(i)
CHAL
1
0.75
0.5
0.25
0
(j)
*
(k)
PAL
0.095
0.07
0.045
0.02
-0.005
(l)
12
72
141
Hours after inoculation
12
72
141
Hours after inoculation

In the present study, higher *MET* expression for plants supplied with calcium silicate at 72 hai may have collaborated to reduce ASR severity. High *MET* expression in soybean plants infected by *Phytophthora sojae* and *Pseudomonas syringae* pv. *glycinea* was linked with their increased resistance against these pathogens (Liu et al., 2001).

The JA spray did not result in differential gene expression (except for PAL at 12 hai) in a scenario where ASR severity was higher. JA-dependent pathway activation in detriment of the SA-dependent pathway was reported for the *Colletotrichum orbiculare*-cucumber and *Fusarium graminearum-Arabidopsis thaliana* interactions (Liu et al., 2008; Makandar et al., 2010) and does not seem to be the case for the soybean–*P. pachyrhizi* interaction.

Chalcone synthase, chalcone reductase and chalcone isomerase are the enzymes involved in the biosynthesis of flavonoids in soybean (Naoumkina et al., 2010). The chalcone isomerase catalyses the stereospecific isomerization of chalcones into their corresponding flavanones (Naoumkina et al., 2010). Plants of a resistant soybean cultivar compared with a susceptible one showed higher concentrations of flavonoids and isoflavonoids and an increase on lignin concentration in response to infection by *P. pachyrhizi* (Lygin et al., 2009). Reduction on ASR symptoms for plants supplied with Si through the roots could be explained by *CHAL* and *PAL* expressions at 12 and 141 hai, respectively.

The *LOX* was differentially expressed for Si-supplied plants through the roots. The production of traumatin, jasmonic acid, oxylipins and volatile aldehydes involved in host defence against different pathogens is dependent on *LOX* expression and high LOX activity (Thakur & Udayashankar, 2019).

Plants of *A. thaliana* supplied with Si and non-inoculated with *Erysiphe cichoracearum* showed alteration in the relative abundance of only two transcripts of a total of 40,000 (Fauteux et al., 2006). In contrast, for inoculated plants, regardless of Si supply, nearly 4,000 genes (host defence genes and genes involved in the primary metabolism were up- and down-regulated, respectively) had their expressions changed. The metabolism of non-inoculated plants supplied with Si did not alter indicating that under fungal infection is when this element plays the most significant role in the modulation of host defence responses (Fautex et al., 2006). In the present study, there was no significant difference in the expression of genes for non-infected plants regardless of Si supply. Chain et al. (2009) studied the effect of Si on wheat plants non-inoculated or inoculated with *Blumeria graminis* f. sp. *tritici* through transcriptomic analysis of 55,000 unigenes. The response of non-inoculated plants supplied with Si was limited to 47 genes of diverse functions with no evidence that a specific metabolic process was regulated. Many genes were up-regulated (involved in stress and metabolic processes) and down-regulated (involved in photosynthesis) for inoculated plants indicating that genes expression modulated by Si was limited in the absence of fungal infection (Chain et al., 2009). A biphasic response of susceptible and resistant soybean genotypes in response to *P. pachyrhizi* infection regarding *PAL* expression occurred at 12 and 72 hai (Mortel et al., 2007).

Tremblay et al. (2010) used laser capture microdissection to isolate susceptible soybean palisade and mesophyll cells infected by *P. pachyrhizi* and to perform a transcriptome analysis. These authors reported that a total of 2982 genes were differentially expressed, of which 685 and 2297 genes were up- (linked to host resistance) and down-regulated (linked to different metabolic pathways), respectively. In the present study, an increase in the expression of defence-related genes on infected plants corroborates with the findings reported by Tremblay et al. (2010). Moreover, it is plausible to postulate that the metabolism of soybean plants may have suffered changes at advanced stages of fungal infection in an attempt to activate defence responses. Furthermore, no biphasic response was noticed on soybean plants infected by *P. pachyrhizi* regardless of Si supply or ASM spray.

Taken together, the results of the present study provide evidence of differential gene expressions involved in the biosynthesis of phenolics (*PAL*) and flavonoids (*CHAL*) in soybean plants supplied with Si to reduce ASR severity.

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ORCID

Maria Fernanda Antunes Cruz https://orcid.org/0000-0003-2305-0767
Fabrício Ávila Rodrigues https://orcid.org/0000-0002-3091-0000

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